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Dear Dr. Boman:

Thank you very much for the preprint of your paper. I enjoyed reading it and was intrigued by the findings and interpretations you made. There is, however, an alternate way of explaining the results. I shall outline it because you may have done or could quickly do the necessary experiments to prove or disprove the hypothesis. The argument goes as follows:

1. Suppose that the S-RNA you used was degraded to the extent that the terminal adenylic acid residue is absent from a major portion of the RNA chains. This is not so far-fetched since this is actually the case in the yeast S-RNA isolated with phenol extraction by Monier and Zamecnik. If this were the case, the only RNA chains capable of accepting any amino acids are those which have the terminal adenylic acid group or those to which the adenylic acid may become linked during the incubation.

2. Consider the proposition that the arginine-activating enzyme is contaminated with non rate-limiting levels of the enzyme which adds AMP residues to the ends of incomplete chains, using ATP as substrate (see Preiss, et al., manuscript 4). Furthermore assume that the yeast "mthionine-activating enzyme" has none or at most a little of this activity. What then are the consequences?

a) The "strange" kinetics can be explained as follows: Methionyl RNA formation starts off rapidly but slows up because the amount of active methionine-specific RNA chains (those having adenylic ends) becomes limiting. This rate would be expected to be markedly increased if by addition of the "arginine-activating enzyme" preparation the inactive chains are converted to active chains by incorporating the terminal AMP residue. No such "strange" kinetics would be expected with the "arginine-activating enzyme" preparation making arginyl RNA or when the methionine- and arginine-activating enzymes are used together. In both of the latter cases all of the RNA chains are converted to competent amino acid acceptors.

b) One would predict that the enzymatic activity lost by exposing the arginine-activating enzyme to pH 4.1 at 0° is the "adenylic acid end group restoring" enzyme. When such an "inactivated" preparation is used the low activity is due to the ability to use only a fraction of the RNA chains (i.e., those with Ad end groups). In this case the addition of AMP to the inactive RNA chains does not occur.

As far as I can tell there is no data in the paper which is in conflict with this idea. It is, however, easily tested. If the above suggestion is correct, pre-incubation of the S-RNA with the "arginine-activating enzyme" preparation, ATP, but no amino acid followed by inacgivation of the added enzyme (by pH 4.1 treatment or by short heating) should yield an RNA which can support rapid methionyl RNA synthesis. Moreover, there should be no effect by the addition of "arginine-activating enzyme". I also think much can be learned about what is happening by examining the yields of methionyl RNA formation in the presence and absence of the "arginine-activating enzyme".

With our preparations of purified E. coli RNA we can load approximately 1.0 mμmole methionine/mg RNA. Judging from Figure 2, it appears that the reaction slows down when about 0.2 mμmole of methione is added per 3.1 mg RNA (or is it per 0.6 mg?). On adding the arginine-activating enzyme it looks like an equivalent number of RNA chains become saturated with amino acid before the rate becomes low.

Observations somewhat similar to the ones you have made can be seen when, for example, one looks at amino acid incorporation using the Monier yeast RNA. Thus there is little or very slow incorporation of amino acid using a purified enzyme but if a system, known to be capable of incorporating AMP, is added the rate and yield increase.

I would like very much to know of your feelings about this suggestion and any data you collect testing the possibilities I have raised.

Very sincerely yours,

Paul Berg

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